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# Acta

## Entomology and Zoology

### A diagnostic study of the cutaneous Leishmania parasite in Salah al-Din governorate

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#### Abstract

The research included the diagnosis of cutaneous leishmaniasis in Salah al-Din Governorate, where the samples included patients visiting Tikrit Teaching Hospital for the period from the beginning of March 2023 until the end of October 2023. The number of patients was 200 patients. The infection was diagnosed by the specialist doctor first, then laboratory diagnosis. (CL) is one of the problems facing patients because it causes deformities at the site of infection. The results of the study showed an increase in the number of infected males, 72.5%, over females, 27.5%, with a large difference. The number of infected patients from rural areas, 70%, was higher than from urban areas, which reached 30%. As for the number of ulcers in the body, the incidence of one ulcer in males was more than in Females accounted for 73.6%, followed by two ulcers. In the case of three ulcers, it amounted to 60%. As for four or more ulcers, one infection was similar in males and females. The ulcers were distributed in the facial areas at a rate of 59.5%, then the lower extremities at 29.5% the upper extremities at 10.5%, and the least in the trunk area, where only one infection was recorded at a rate of 0.5%. The study showed that the highest infection rate was 76% of patients with wet ulcers, while the incidence of ulcers was recorded. Dry by less than 24%.

**Keywords:** Cutaneous leishmaniasis, amastigote, sand fly

#### Introduction

Leishmaniasis is a parasitic disease of zoonotic origin. It is caused by parasitic protozoa that humans infect through the bite of a female sand fly (*Phlebotomus papatasi* and *P. sergenti* species). This insect is small in size and lives in hot] humid weather. Its activity increases in the summer. It feeds on the blood of humans and animals <sup>[1]</sup>. Cutaneous Leishmaniasis which infects humans, is caused by a parasite of the *L. tropica* type, which spreads in large areas in Baghdad and Mosul governorates due to the poor health conditions of the population, while the *L. major* type is more widespread in rural areas in the southern and northern governorates of our country <sup>[2]</sup>. Cutaneous leishmaniasis or Oriental sore, also called Baghdad's boil, is an endemic disease in Iraq as well as in other countries such as Iran, Kuwait, and Afghanistan <sup>[3]</sup>. The Leishmania parasite lives inside the phagocytic cells of the vertebrate host in the amastigote form and in the intestine of the sand fly insect in the promastigote form <sup>[4]</sup>.

#### Materials and Methods

**Sample collection:** The study included 200 people infected with (CL) after it was diagnosed by doctors specializing in dermatology at Tikrit Teaching Hospital for the period from March 2023 until October 2023. Complete information was recorded by the patient in a form specifically for (CL).

**Isolation of Leishmania parasites:** (CL) parasites were isolated from the skin lesion after the specialist physician diagnosed them according to the method of <sup>[5]</sup>. The skin area was cleaned well with ethyl alcohol at a concentration of 70%, then left to dry, and a one-ml wine syringe containing (0.1- 0.2) ml of sterile saline solution under the skin at the pink edge of the skin surrounding the ulcer, then rotating the needle (2-3) times while it is in the skin, cutting small pieces of tissue from the edge of the wound to the needle, while isolating the (CL) parasite. With drawing the injection, I took a drop of blood from the area of the stitches on a clean glass slide and made a smear that was fixed with ethyl alcohol at a concentration

of 95% and stained with Giemsa stain to confirm the presence of the a mastigote stage of the Leishmania parasites [6].

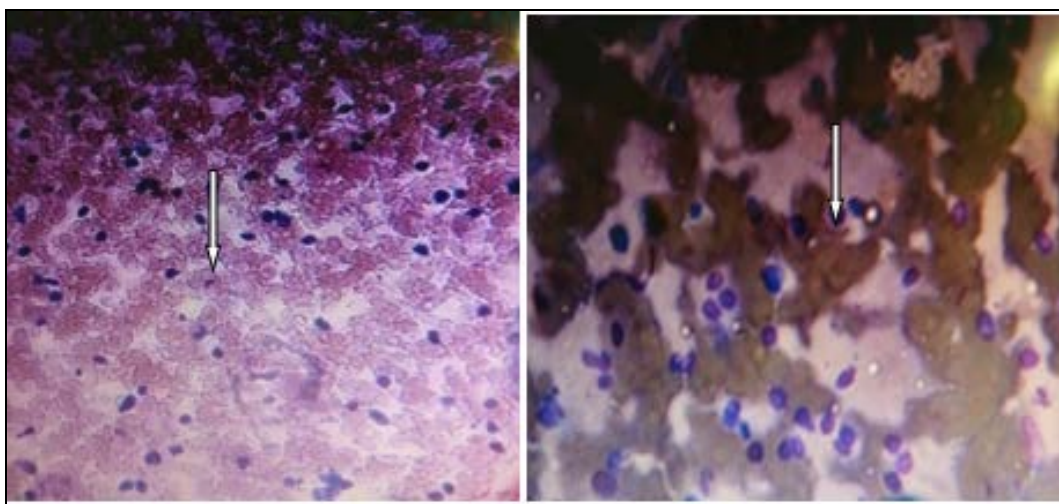
**Staining:** Giemsa stain was used to stain the parasite according to the method of [7]. This stain is an easy to prepare dye and consists of 3.8 grams of Giemsa stain powder, 250 cm<sup>3</sup> of glycerol, and 250 cm<sup>3</sup> of methanol, which we mix the materials are placed in a suitable bottle and shaken vigorously, then incubated at 37 °C for 24 hours, after which the dye is placed in a Shaker incubator for several hours until it is ready for use. It is preferable to filter it before use.

**Staining method:** Prepare a thin swab of parasites by placing a small drop of the solution containing the parasites near the edge of a clean glass slide and spreading it using the edge of another glass slide, then leaving it to dry. Fix the

swab by placing a few drops of methanol alcohol on it for one minute. Dilute the dye with buffer solution (PBS) at a rate of 15% and place it in the container designated for dyeing. Then we immerse the glass slide in it and leave it for 15 minutes. Remove the slide and wash it with buffer solution or tap water. Leave the slide to dry and then examine it under a microscope using an oil lens [6].

**Results**

Isolation of (CL) from skin ulcers in infected patients infection with (CL) was detected, as the results showed 200 cases of cutaneous leishmaniasis in Tikrit Teaching Hospital from patients coming from different areas of Salah al-Din. The samples were examined laboratory under a microscope using an oil lens after the infection was diagnosed by the specialist doctor.



**Fig (1):** A blood smear showing the amastigote stage of leishmaniasis in humans in Table (1) The results showed a higher infection rate in males than in females. The results showed that there were differences between the sexes, and the majority of those infected with (CL) were males in the age groups (11-15) years, at a rate of 32.5% and the lowest rate was in the ages less than 50 years at a rate 0.5%.

**Table 1:** Distribution of (CL) among infected people based on age and sex

Age groups	Males	Females	Total	%Percentage
1-5	8	4	12	6%
6-10	39	11	50	25%
11-15	45	20	65	32.5%
16-20	30	10	40	20%
21-25	7	4	11	5.5%
26-30	5	2	7	4.5%
31-35	4	2	6	3%
36-40	4	1	5	2.5%
41-45	2	1	3	1.5%
46-50	1	0	1	0.5%
Total	145	55	200	100

The distribution of infection cases based on the type of residence showed that the disease was evident in 60 patients 30% from urban areas, and in 140 patients 70% from rural areas, with a large difference according to the type of residence. As for the months of the year, the highest infection rate was in April and May, and the lowest in October.

**Table 2:** Distribution of cutaneous leishmaniasis infection based on type of residence, sex, and months of the year.

Month	Rural areas		Urban areas	
	Males	Females	Males	Females
March	21	5	2	1
April	19	13	5	2
May	14	7	9	2
June	10	5	4	1
July	13	6	13	3
August	8	5	9	1
September	9	3	5	0
October	2	0	2	1
Total	140 (70%)		(30%) 60	

The results showed that there were differences in the number of skin ulcers between males and females and in the number of ulcers. Infection with one ulcer was more common in males than in females at a rate of 81%, then two ulcers at a rate of 53%. As for infection with three or four ulcers, it was similar in females and males as in Table (3).

**Table 3:** Distribution of the incidence of (CL) relative to the number of skin ulcers.

Gender	Number of ulcers				Total
	1	2	3	4	
Male	81(73.6%)	53(72.6)	9(60%)	2(100%)	145
Females	29(26.3%)	20(27.3%)	6(40%)	0(0%)	55
Total	110	73	15	2	200

**Distribution of (CL) infection based on body regions**

Table (4) shows a study where the infection was concentrated in the face, where the number of lesions reached 59.5%, followed by the lower extremities at 29.5%, then the upper extremities at 10.5%. As for the trunk, the results showed that there was only one infection case at a rate of 0.5%.

**Table 4:** Distribution of (CL) infection among expatriates according to body regions.

Location of ulcer	Male	Female	Total	Percentage%
The face	79	40	119	59.5%
The upper extremities	12	9	21	10.5%
The Lower extremities	53	6	59	29.5%
The trunk	1	0	1	0.5%
The total	145	55	200	100

The study showed that 152 patients had wet ulcers, accounting for 76%, while 48 dry ulcers were reported, accounting for 24% of the patients, as in Table 5.

**Table 5:** Distribution of cutaneous leishmaniasis based on the type of skin ulcer

Type of ulcer	Male	Female	Total	Percentage%
Wet ulcer	113	39	152	76%
Dry ulcer	32	16	48	24%
Total	145	55	200	100

**Discussion**

The diagnosis of (CL) is based on microscopic examination of Giemsa-stained smears and parasite samples isolated from skin lesions from patients suspected of having CL, and this is consistent with [8] showed that the diagnosis of leishmaniasis depends on detecting the a mastigotes stage in the smear, and this depends on the number of parasites and the experience of the person examining the slide. The incidence of (CL) in males was higher than in females, and this percentage is consistent with the findings of [9] in Iraq. The reason may be attributed, according to [10]. To the fact that male children tend to play outside the home more than females, and the clothes they wear are exposed at the legs and hands. The infection is less among females. This may be due to the lack of outdoor activities for females in our society, and closed clothing is one of the factors that contribute to the decrease in the number of infection cases [11].

The results of the research showed that the highest infection rate according to age groups was among those aged [10-15] years, and the lowest rate was in the age group less than 50 years. These results were close to the findings of [12]. Who indicated that older children More susceptible to infection. This result was also identical to a study in Iran, in which the infection rate was 31.6% in the age group of (10-15) years

[13, 9] the reason was attributed to the fact that the immune system is less developed in younger children compared to other age groups, which makes them more susceptible to infection [14]. Males also come into contact with sand fly vectors [11]. Although sex hormones may influence the disease course of the parasite, behavioral factors make males more susceptible to vectors in the environment [15].

As for residential areas, the infection rate has increased in rural areas and is higher than in urban areas. This high rate may be attributed to younger children, especially In rural areas like our city the rural population outnumbers the urban population. Regarding infection and the number of ulcers. The highest rate was one ulcer which is common, and it was consistent with [16]. The presence of several ulcers was attributed to exposure to a number of bites by infected insects. As for the distribution of skin ulcers on the bodies of those affected, the face includes the largest number of infections, as the face was first, followed by the lower extremities, upper extremities, and torso, with only one infection. The study showed a clear difference between the wet type and the dry type, which is attributed to the factors causing it and the presence of a secondary infection that gives the lesion an ulcerative appearance. The result was identical with [17]. In Iraq who stated that the wet type is more widespread than the dry type, and that the disease is higher in areas rural areas due to exposure to the vector and reservoir hosts, which are considered the causative agents of the disease in these places where the disease is endemic.

**Conclusion**

In conclusion, the diagnosis of cutaneous leishmaniasis (CL) relies on microscopic examination of Giemsa-stained smears and parasite samples obtained from skin lesions, consistent with previous studies. The higher incidence of CL among males compared to females could be attributed to behavioral factors such as outdoor activities and clothing practices. Younger age groups, particularly those aged 10-15 years, showed the highest infection rates, likely due to their less developed immune systems and increased exposure to sand fly vectors. Additionally, the infection rate was found to be higher in rural areas compared to urban areas, possibly due to the higher population density of susceptible individuals in rural settings. The distribution of skin ulcers primarily on the face, followed by lower and upper extremities, correlates with previous findings and suggests differential susceptibility to the disease based on exposure. Furthermore, the distinction between wet and dry types of CL reflects variations in disease presentation and the presence of secondary infections, with wet type being more prevalent in rural areas where vector and reservoir hosts are abundant. Overall, these findings underscore the importance of understanding epidemiological factors and local conditions in the management and prevention of CL.

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